ELECTRON MICROSCOPE STUDY OF THROMBOCYTES IN ACUTE LEUKOSIS IN CHILDHOOD

S. Kertikova, S. Dimitrov

The hemorrhagic syndrome in acute leukosis is conditioned equally by the reduced number of thrombocytes, and by disturbances in their structure and functional patterns. The earliest electron-microscopic descriptions of thrombocytes in leukosis patients, made by Rebuck, Ridle, Monto (cited by Schultz — 7), date back to 1961. In a great number of cases, particularly in chronic leucosis, considerable ultrastructural variations have been observed in the thrombocytes, manifested with proliferation of endoplasmic reticulum, γ-granulomere hyperplasia, and thrombocyte membrane disorders. On the other hand, in experimental Malonney-leukosis, Malonney and Dalton (1962) (cited by Schultz — 7) succeeded in detecting, at electron microscope level, viruses localized in special intracytoplasmic vacuoles.

In 1967, Kirsten (cited by Schultz — 7) discovered the erythroblastosis virus on the thrombocytic membrane of mice, and in the hyalomere of mice thrombocytes.

Irrespective of the above reports, the interest in the ultrastructure of thrombocytes in acute leukosis continues, and already for two decades, complex histologic, autoradiographic, immunofluorescence and electron microscope studies are in course.

Using differential ultracentrifugation of thrombocytic suspensions in media with different gradient density, it was established that by their ultrastructural characteristic features, the thrombocytes are differentiated into thrombocyte population types — A, B, C and D.

The thrombocytes of the A and B population represent thrombocytes with relatively low-density gradients: population A consist of small thrombocytes, deprived of granules, some of them with preserved as yet osmiophilic, dense granules whereas others are absolutely empty — the so-called thrombocytic shadows; the population B thrombocytes are with heterogeneous morphologic appearance, rather frequently damaged, with pronounced vacolization and pseudopodia; the thrombocytes of population C display clearcut morphology with occasional ovoid forms being visible; they too are heterogeneous morphologicalwise, but the intracytoplasmic granular content is preserved. The most intact morphology is disclosed by the population D thrombocytes — it is a matter of functionally young, very active thrombocytes with a rich granular and microtubular content.

During the leukosis prosess, the changes in thrombocyte metabolism influence the thrombocytic populations' profile.

We undertook the task to study the fine structure of thrombocytes in acute leukosis among children.

Material and method

A series of fifteen children affected with acute lymphoblasitic leukosis, aged 2 years and 4 months to 14 years before initiating the treatment, were investigated. Using silicon technique, 4.5 cc venous blood was obtained from each patient, with 0.5 cc anticoagulant—3.8 per cent sodium citrate. Following spontaneous blood sedimentation, the rich in thrombocytes plasma underwent centrifugation at 3 000 rev for ten minutes. Next, threefold rinsing of the thrombocytes with physiological saline was made. The thrombocytes thus washed were fixed in 4 per cent glutaraldehyde, in phosphate buffer at pH 7.3 for forty minutes, and thereafter — in 2 per cent OsO_4 for 90 minutes. After subsequent dehydration in graduated alcohols, the material was embedded in durcupan ACM. Ultrastructural sections were prepared in ultramicrotome Reichert, while contrast staining was carried out according to Reynolds with uranyl acetate and lead nitrate. The material was studied in electron microscope JEM — 7a.

Results

Thrombocytopenia was established in all the children under study, with the thrombocytic count ranging from 10 000 to 85 000.

The electron microscope structure of thrombocytes shows characteristic pathological variations. The form of thrombocytes is usually round-oval or uneven, with single pseudopodia along the surface. In most of the thrombocytes, the thrombocytic membrane is poorly delineated and even lacerated in some places. Elsewhere, practically uniform thickening of the thrombocytic membrane can be seen, with particularly clearcut electron-bright transitional layer.

A different electron density is characteristic of the hyalomere. Often, thrombocytes with strongly transparent hyaloplasm are noted. In other instances, nearby the thrombocytic membrane single microtubules are present. Ribosomes are very well represented in singles or in groups, scattered within the hyaloplasm, and forming polyribosomes in some places. In part of the thrombocytes, lipid inclusions are also observed.

The granulomere as well shows a number of pathologic variations. α-granulomere is considerably reduced. Aggregation of granules occurs in one or both poles of the cell. Their number relative to normal human thrombocytes is strongly diminished. In isolated thrombocytes, 1—2 α-granules only can be seen. In other cases variations in the form and size of α-granules are established. Giant-size granules with elipsoid or pear-like shape, displaying a high electron density, are met with. At times, the α-granule is bilaterally thickened in a bat-like pattern.

For the most part, the β-granulomere is very well manifested. Whilst in normal thrombocytes the alpha-beta ratio is 4:1, here it is virtually just the opposite. The number of mitochondria with irregular shape and ununiform disposition of the mitochondrial cristae augments. Not infrequently, the cristae are very dense or notched. Part of the mitochondria have already undergone vacuolization.

The γ-granulomere shows heavy hypertrophy and in some places it forms large vesicles. In the latter instance, the other elements of the granulomere are strongly reduced (Fig. 1).

Thrombocytes with a σ-granulomere were not encountered in the cases observed.

The Σ-granulomere is variably represented. In most cases it is a matter of a strongly reduced, or practically absent glycogen. In other glycogen packages or diffusely scattered glycogen granules occur.

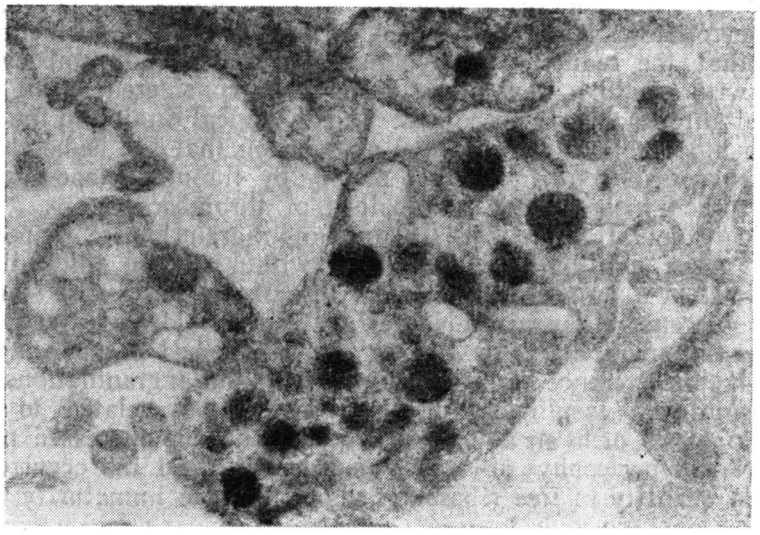

Fig. 1. Thrombocytes with Σ-granulomere hypertrophy, magnific. 20 000 1.5 x.

Discussion

The ultrastructure of thrombocytes in acute leucosis among children is substantially altered with all structural components being involved. The observations disclose a notched or moderately thickened thrombocytic membrane, increased transparence of the hyalomere with increase in the number of free ribosomes, appearance of lipid inclusions, α— granulomere reduction or pathologic α-granules: pear-shaped, ellipsoid or bilaterally thickened in a bat-like fashion, increase and hypertrophy of mitochondria with uneven build-up of the mitochondrial cristae and vacuolization, strongly manifested hypertrophy of the γ-granulomere, often met with Σ-granulomere reduction.

The above described morphological changes suggest that in acute leukosis, the thrombocyte populations of the A and B types prevail, that is, old functionelly inactive cells, with strongly reduced enzyme activity. Similar pathologic variations in the structure of thrombocytes in adult patients with acute-leucosis were discovered by Dobreva and Genova (2, 3, 4), Alkerman and Bezsonnikov (sidet by Alexiev — 1). In the course of electron microscope study on thrombocytes in a series of 49 patients with various forms of leukosis the authors referred to succeeded in establishing a considerable reduction of mature thrombocyte forms in the acute period of the disease, as well as a rise in the quantity of degenerative and old thrombocytes, and enhanced vacuolization. Upon appearance of hematologic remission, Sofaryan and Izmailova (cited by

Alexiev) observed an increase in the quantity of mature thrombocytes, although they failed to detect a complete thrombocytogram normalization. On the other hand, the presence of intrathrombocytic lipid formations similary ints to the presence of profound metabolic derangements: Jean, Racine, Marx, Gauier (5, 6) studied the lipid formations in patients with various thrombocytopathies and in healthy persons, and were successful in demonstrating that in normal thrombocytes practically no lipid formations occur, whereas in thrombocytopathies their quantity augments substantially; furthermore, they point out that the reduction of glycogen quantity in the thrombocyte simultaneously with steatosis development might be considered as an indicator of disturded thrombocyte metabolism. The analogical finding in the thrombocytes in acute leukosis warrants the assumption that the hemorrhagic syndrome in leukosis is conditioned not merely by thrombocytopenia, but also by the disturbed intermediary metabolism of thrombocytes.

Pathologic deviations in the structure of α-granulomere (appearance of giant α-granules, ellipsoid, pear-shaped or with a bat-like thickening) have been described in a number of congenital thrombopathies — the syndrome of Willebrand-Jurgens, Glanzmann-Naegeli, Wiscott-Aldrich — with a deficiecny of the thrombocytic factor 3 being also established in most of the cases. Consequently, the presence of thrombocytes with similar granulations in acute leukosis points to insufficiecy in their maturation, related to defective thrombocytopoiesis, or to an abnormal acceleration. The increased number of mitochondria, hypertrophy of the γ-granulomere, and the occurrence of a considerable quantity of free ribosomes also attest the immaturity of thrombocytes.

On the ground of the studied electronograms of thrombocytes in acute childhood leukosis, the following inferences might be reached:

1. Electron-microscopically, a considerable reduction of the α-granulomere or occurrence of pathological granules is observed in the thrombocytes. The mitochondria are numerous, often vacuolized or displaying an irregular disposition of the mitochondrial cristae. The γ-granulomere has undergone strong hypertrophy, and in sone places it forms large vacuoles. Lipid formations are noted in part of the thrombocytes.
2. The pathologic variations established in the ultrastructure of thrombocytes point to heavy disturbances in thrombocyte metabolism which, along with thrombocytopenia or independently, conditions the hemorrhagic syndrome in the acute stage of leukosis.

REFERENCES

1. Алексеев, Н. А. *Педиатрия,* 1963, 10. — 2. Генова, В. Енергиен метаболизъм, патофизиология на тромбоцитите и тромбцитни популации при левкозите в Левкозния проблем, стр. 40—55, 1975 София. — 3. Добрева, А., В. Генова. Ултраструктура на тромбоцитите при острата левкоза» — доклад, изнесен на I национална конференция по електронна микроскопия, Варна, IX. 1971. — 4. Добрева, А. Ултраструктура на нормалните човешки тромбоцити. *Съвременна медицина,* бр. 11, 1971. — 5. Jean, G., Racine, L., Marx, R., R. Gautier, A. *Thromb. Diath. Haemorrh.* (Stuttgart), 10, 42, 1963. — 6. Jean, G., Racine, L., Gautier, A., Marx, R. *Thromb. Diath. Haemorragica* (Stuttgart), 10, 9, 1, 1963. — 7. 7. Schultz, H. Thrombocyten und Thrombose in Electronenmicroscopischen Bild, 1968.

ЭЛЕКТРОННОМИКРОСКОПИЧЕСКИЕ ИССЛЕДОВАНИЯ ТРОМБОЦИТОВ ПРИ ОСТРОМ ЛЕЙКОЗЕ В ДЕТСКОМ ВОЗРАСТЕ

С. Кертикова, С. Димитров

РЕЗЮМЕ

Геморрагический синдром при остром лейкозе обусловлен чаще всего уменьшением числа тромбоцитов, нарушением их структуры и функциональных отправлений.

Авторы исследуют ультраструктуру тромбоцитов в периферической крови у 10 дедей с острым лейкозом. Устанавливаются значительное редуцирование α-грануломеров или появление паталогической грануляции — гигантских или неправильной формы, β-грануломер в большинстве случаев сильно развит, в то время как часть митохондрий с патологическими отклонениями, γ-грануломер резко гипертрофирован и на местах образуются большие везикулы. В части тромбоцитов наблюдаются жировые включения.